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(54) NUTRIENT MEDIA FOR
 MICROBIOLOGICAL TESTING

- (71) We, BOEHRINGER INGELHEIM G.m.b.H. a Body Corporate organized under the laws of the Federal Republic of Germany, of Ingelheim am Rhein, Federal Republic of Germany do hereby declare the invention, for which we pray that a patent may be granted to us, and the method by which it is to be performed, to be particularly described in and by the following statement:-
- The present invention relates to a method of determining the genera of pathogens capable of causing infections of the urogenital tract and to a kit comprising certain nutrient media for use in the determination of the genera of such pathogens.
- Methods and kits for determining the nature of microorganisms in various test materials have been described, for example, in German Offenlegungsschrift No. 2,408,167. The methods and kits described hereto, however, have involved the use of known nutrient media, and the selectivity of these methods and kits have not been sufficient to enable the desired determination of the genera of pathogens.
- The methods and kits of the present invention avoid, at least in part, the disadvantage of insufficient selectivity.
- According to one feature of the present invention there is provided a method for determining the genera of pathogens capable of causing urogenital tract infections which comprises inoculating each of the following nutrient media:
- Lactose-P-Nutrient (as herein defined),
 - Citrate-G-Nutrient (as herein defined),
 - Cadmium Nutrient (as herein defined),
 - Phenylalanine-lithium-G-Nutrient (as herein defined),
 - DNase Nutrient (as herein defined),
 - Mannitol-Thiocyanate Nutrient (as herein defined),
 - T.T. Nutrient (as herein defined),
 - Peptone-Thiocyanate Nutrient (as herein defined)
- with a sample of the said pathogen, incubating each of the nutrient media, and determining the genera of the said pathogen by evaluation of the growth if any on each of the nutrient media.
- The nutrient media designated:- citrate-G-nutrient, phenylalanine-lithium-G-nutrient, mannitol-thiocyanate nutrient and peptone-thiocyanate nutrient are novel *per se* and have not, hitherto, been described in an equivalent or similar composition. The remaining nutrient media are based on known media modified by altering the quantities of the components and/or adding additional substances.
- The method of the present invention, enables those pathogens which cause a very high percentage of urogenital infections (*Escherichia*, *Proteus*, *Klebsiella*, *Enterobacter*, *Pseudomonas*, *Serratia*, *Providencia*, *Citrobacter*, *Staphylococcus*, *Streptococcus*, *Candida*) to be quickly and accurately identified. Moreover with regard to sensitivity determination according to German Offenlegungsschrift P 2,301,211, the method according to the invention provides a basis for successful therapy.
- The microorganisms may, if required, be examined by means of simple trials.
- The composition, preparation and characteristics of the eight nutrient media are described herein. Deviations from the indicated compositions may be made if these do not impede selectivity and growth of the microorganisms.
- The term "Lactose-P-Nutrient" as used herein denotes a nutrient medium comprising

- lactose, an indicator such as bromothymol blue, an anionic surfactant in a concentration effective to inhibit the growth of Gram-positive micro-organisms, and pimarinine in a concentration effective to inhibit the growth of *Candida*. Using bromothymol blue as the indicator the composition of the medium is such that pathogens of the genera *Citrobacter* 5 incubated on the said medium produce a blue to blue-green colouration, pathogens of the genera *Enterobacter* incubated on the said medium produce a yellowish green colouration and pathogens of the genera *Escherichia* incubated on the said medium produce a yellow colouration. Other indicators of equivalent pH range can be used instead of bromothymol blue.
- 10 The surfactant employed in Lactose-P-Nutrient (as hereinbefore defined) is preferably an anionic long chain alkyl sulphate e.g. heptadecyl sulfate preferably used as its sodium salt (Tergitol 7; "Tergitol" is a registered Trade Mark). In one preferred embodiment "Lactose-P-Nutrient" comprises lactose, polypeptone, yeast extract, sodium heptadecyl sulfate, pimarinine, bromothymol blue, agar-agar and distilled water. In one especially preferred 15 embodiment "Lactose-P-Nutrient" has the following composition:-
- | | | | |
|----|--|---------------|----|
| | Lactose | about 10.0 g | |
| | polypeptone | about 5.0 g | |
| | yeast extract | about 3.0 g | |
| 20 | sodium heptadecyl sulfate (Tergitol 7) | about 0.15 g | 20 |
| | pimarinine | about 0.1 g | |
| | bromothymol blue | about 0.025 g | |
| | agar-agar | about 18.0 g | |
- 25 per litre of deionised or distilled water. The nutrient medium has a pH of 6.9 ± 0.1 . Sodium heptadecyl sulfate in the indicated concentration inhibits the growth of the Gram-positive microorganisms, e.g. *Staphylococcus* and *Streptococcus*. Pimarinine in the mentioned concentrations inhibits the growth of *Candida* and other yeasts and mould fungi.
- 30 The term "Citrate-G-Nutrient" as used herein denotes a nutrient medium comprising a water-soluble citrate, bile salts in a concentration effective to inhibit the growth of gram-positive microorganisms, an indicator dye such as phenol red and pimarinine in a concentration effective to inhibit the growth of *Candida*; the composition of the medium being such that pathogens of the genera *Citrobacter* *Enterobacter* and *Klebsiella* incubated on the said 35 medium produce a visible colour change, for instance a raspberry red colouration when phenol red is the indicator.
- Citrate-G-nutrient (as hereinbefore defined) preferably also contains glucose and yeast extract in a concentration effective to shorten the growth-lag-phase and quicken the break down of citrate. In general the use of yeast extract and glucose enable evaluation to take 40 place within 24 hours.
- The water soluble citrate employed is conveniently sodium or potassium citrate e.g. trisodium citrate dihydrate.
- In a preferred embodiment "Citrate-G-nutrient" comprises trisodium citrate dihydrate, a mixture of salts of bile acids, pimarinine, glucose, yeast extract, sodium chloride, hydrated 45 magnesium sulfate, ammonium dihydrogen phosphate, hydrated sodium ammonium hydrogen phosphate, phenol red, agar-agar and deionised or distilled water. In one especially preferred embodiment of the present invention "Citrate-G-nutrient" has the composition:-
- | | | | |
|----|---|--------------|----|
| | Trisodium citrate dihydrate | about 5.0 g | |
| 50 | mixture of salts of bile acids | about 1.0 g | 50 |
| | pimarinine | about 0.1 g | |
| | glucose | about 0.05 g | |
| | yeast extract | about 0.25 g | |
| | sodium chloride | about 5.0 g | |
| 55 | magnesium sulfate heptahydrate | about 0.2 g | 55 |
| | ammonium dihydrogen phosphate | about 0.2 g | |
| | sodium ammonium hydrogen phosphate tetrahydrate | about 0.8 g | |
| | phenol red | about 0.02 g | |
| 60 | agar-agar | about 15.0 g | 60 |
- per litre of deionised or distilled water. The nutrient medium has a pH of 7.1 ± 0.1 . The mixture of salts of bile acids in the abovementioned concentration inhibits the growth of Gram-positive microorganisms, namely *Staphylococcus* and *Streptococcus*. 65 Pimarinine in the above-mentioned concentration inhibits the growth of *Candida* and

other yeasts and mould fungi.

In combination with "Lactose-P-Nutrient" and the mobility test the pathogen genera *Citrobacter*, *Enterobacter*, *Escherichia* and *Klebsiella* may be distinguished from one another.

5 The term "Cadmium nutrient" as used herein denotes a nutrient medium comprising N-alkyl-aminocrotonic acid and a water soluble cadmium salt in a concentration effective to inhibit the growth of Gram-positive bacteria and *Candida* but to promote the formation of pyoverdin (fluorescent pigments) on the growth of *Pseudomonas*; the composition of the medium being such that pathogens of the genera *Pseudomonas* incubated on the said medium produce a green to yellow colouration. 5

10 The water soluble cadmium salt used is preferably cadmium sulfate. In a preferred embodiment "Cadmium nutrient" comprises preteose peptone, sodium chloride, dipotassium hydrogen phosphate, potassium nitrate, hydrated calcium nitrate, hydrated magnesium sulfate, hydrated cadmium sulfate, glycerin, an N-alkyl-aminocrotonic acid, agar-agar and deionised or distilled water. In one especially preferred embodiment "Cadmium nutrient" has the composition:- 10

	Proteose peptone	about 20.0 g	
	sodium chloride	about 1.2 g	
20	dipotassium hydrogenphosphate	about 0.8 g	20
	potassium nitrate	about 0.6 g	
	calcium nitrate tetrahydrate	about 0.4 g	
	magnesium sulfate heptahydrate	about 0.6 g	
	cadmium sulfate octahydrate	about 0.005 g	
25	glycerin	about 8.0 ml	25
	N-alkyl-aminocrotonic acid	about 0.5 g	
	agar-agar	about 15.0 g	

30 per litre of deionised or distilled water. The nutrient medium has a pH of 7.2 ± 0.1 . The N-alkyl-aminocrotonic acid for use in the "cadmium nutrient" medium of the present invention is preferably that available under the trade mark "Ampholyte KKPK 55". 30

N-alkyl-aminocrotonic acid and water soluble cadmium salts, e.g. cadmium sulfate, in the above-indicated concentrations will inhibit Gram-positive and most Gram-negative bacteria as well as *Candida* and other yeasts and mould fungi. Of the Gram-negative bacteria, that may grow on this nutrient medium, only *Pseudomonas* is in a position to form pyoverdin (fluorescent pigments). 35

The above-mentioned composition of the nutrient medium enhances formation of pyoverdin and enables *Pseudomonas aeruginosa* to be identified within 24 hours.

40 The term "Phenylalanine-lithium-G-nutrient" as used herein denotes a nutrient medium comprising salts of bile acids in a concentration effective to inhibit the growth of Gram-positive microorganisms, pimaricine in a concentration effective to inhibit the growth of *Candida*, a water soluble lithium salt in a concentration effective to reduce the growth of *Enterobacteria* other than *Proteus* and *Providencia* and phenylalanine in a concentration effective to allow conversion into phenylpyruvic acid by *Proteus* and *Providencia*; the composition of the medium being such that pathogens of the genera *Proteus* and *Providencia* incubated on the said medium produce a green colouration on the addition of a solution of a ferric salt. 45

50 Phenylalanine employed in "Phenylalanine-lithium-G-nutrient" is DL or more preferably L-phenylalanine and the water soluble lithium salt may, for example, be lithium chloride. The solution of a ferric salt employed to test for *Proteus* and *Providencia* may, for example, be ferric chloride. 50

55 In a preferred embodiment "Phenylalanine-lithium-G-nutrient" comprises proteose peptone, yeast extract, sodium chloride, disodium hydrogen phosphate, potassium hydrogen phosphate, L-phenylalanine, a mixture of salts of bile acids, lithium chloride, pimaricine, agar-agar and deionised or distilled water and in an especially preferred embodiment has the composition:- 55

	Proteose peptone	about 10.0 g	
	yeast extract	about 3.0 g	
60	sodium chloride	about 5.0 g	60
	disodium hydrogen phosphate	about 5.6 g	
	potassium hydrogen phosphate	about 0.2 g	
	L-phenylalanine	about 2.0 g	
	mixture of salts of bile acids	about 1.0 g	
65	lithium chloride	about 2.0 g	65

pimaricine about 0.2 g
 agar-agar about 16.0 g

per litre of deionised or distilled water. The nutrient medium has a pH of 7.2 ± 0.1 .
 5 A mixture of salts of bile acids in the above-mentioned concentration inhibits the growth of Gram-positive microorganisms.

Pimaricine in the above-indicated concentration inhibits the growth of *Candida* and other yeasts and mould fungi.

10 Water soluble lithium salts e.g. lithium chloride reduces the growth of most of the Gram-negative Enterobacteria, other than *Proteus* and *Providencia*. *Proteus* and *Providencia* alone are in a position to convert phenylalanine into phenylpyruvic acid. After addition of a water soluble ferric salt such as ferric chloride e.g. several drops of a 10% iron (III) - chloride solution a green colour appears (phenylalanine-desaminase test).

15 The term "DNase nutrient" as used herein denotes a nutrient medium comprising DNase and N-alkyl-aminocrotonic acid in a concentration effective to inhibit the growth of Gram-positive microorganisms, yeasts and mould-fungi; the composition of the medium being such that pathogens of the genus *Serratia* incubated on the said medium produce a clear zone around the area of growth surrounded by a turbid precipitation on the addition of dilute hydrochloric acid e.g. 1N HCl.

20 The "DNase nutrient" (as hereinbefore defined) may, if desired, include an indicator such as methyl green, in which case the test for *Serratia* with dilute hydrochloric acid may be dispensed with.

25 In a preferred embodiment "DNase nutrient" comprises tryptose, desoxyribonucleic acid, sodium chloride and N-alkyl-aminocrotonic acid, agar-agar and deionised or distilled water and in an especially preferred embodiment has the composition:-

	Tryptose	about 16.0 g	
	desoxyribonucleic acid	about 1.6 g	
	sodium chloride	about 4.0 g	
30	N-alkyl-aminocrotonic acid	about 0.15 g	30
	agar-agar	about 12.0 g	

per litre of deionised or distilled water. The pH of the nutrient medium is about 7.3. If desired methyl green may be included in the nutrient medium in a concentration of about 0.04 g per litre of distilled water.

35 The N-alkyl-aminocrotonic acid for use in the "DNase nutrient" medium of the present invention is preferably that available under the trade mark "Ampholyte KKPK 55".

The N-alkyl-aminocrotonic acid in the above-mentioned concentration inhibits the growth of the Gram-positive microorganisms yeasts and mould-fungi.

40 Only *Serratia* of all the pathogens of urinary infections may grow on this nutrient medium and is capable of attacking DNase and of producing a turbid precipitation after the addition of dilute hydrochloric acid e.g. 1 N hydrochloric acid.

The term "Mannitol-thiocyanate nutrient" as used herein denotes a nutrient medium comprising mannitol and a water-soluble thiocyanate. The "Mannitol-thiocyanate nutrient" preferably also contains a water soluble lithium salt, a water soluble potassium salt and a water soluble chloride in a concentration effective to inhibit the growth of flora accompanying the pathogen but not effective to reduce the growth of *Staphylococci*, pimaricine in a concentration effective to inhibit the growth of *Candida*; the composition of the medium being such that pathogens of the genus *Staphylococci* incubated on the said medium produce acid detectable by a pH indicator.

45 The water soluble lithium salt may, for example, be lithium chloride, the water soluble potassium salt may, for example, be potassium chloride, the water soluble chloride may, for example be potassium and/or sodium chloride and the water soluble thiocyanate may for example, be potassium thiocyanate.

55 In order to measure the pH change resulting from the production of acid in the nutrient medium it is convenient to add a pH indicator e.g. phenol red to the nutrient medium. It is also possible to flood the incubated culture with an indicator solution.

60 In a preferred embodiment "Mannitol-thiocyanate nutrient" comprises casein peptone, meat extract, gelatin, D-mannitol, yeast extract, sodium pyruvate, disodium hydrogen phosphate, lithium chloride, sodium chloride, potassium thiocyanate, a pH indicator, pimaricine, agar-agar and deionised or distilled water and in an especially preferred embodiment has the composition:-

65	Casein peptone	about 10.0 g	65
	meat extract	about 5.0 g	

	gelatin	about 5.0 g	
	D-mannitol	about 10.0 g	
	yeast extract	about 5.0 g	
	sodium pyruvate	about 10.0 g	
5	disodium hydrogen phosphate	about 6.0 g	5
	lithium chloride	about 5.0 g	
	sodium chloride	about 5.0 g	
	potassium thiocyanate	about 30.0 g	
	pH indicator	about 0.02 g	
10	pimaricine	about 0.2 g	10
	agar-agar	about 16.0 g	

per litre of deionised or distilled water. The pH of the nutrient medium is about 7.2. If desired the pH indicator may be phenol red.

15 With regard to the preferred embodiment above-described lithium chloride, sodium chloride and potassium thiocyanate in the above-indicated concentrations inhibit the growth of the flora accompanying the pathogen without exercising a negative influence upon the growth of the *Staphylococci*. 15

20 The mannitol-positive *Staphylococci* grow within 24 hours, and the break down of mannitol to give acid may, for example, be indicated by a change in the pH-indicator (e.g. phenol red). Where phenol red is used as indicator the nutrient medium becomes reddish-yellow to yellow. The mannitol-negative *Staphylococci* grow in smaller colonies and the nutrient medium does not show any change in colour. Thus it is possible to note a difference between microorganisms of the *Staphylococci* genera. The pathogenic species *Staphylococcus aureus* is mannitol-positive, while biotypes of the mostly non-pathogenic species *Staphylococcus epidermidis* and *Staphylococcus saprophyticus* are mannitol-negative. 25

The above described nutrient media do not contain substances which deteriorate rapidly or which can only be stored for a short time. The nutrient medium has, as opposed to known nutrient media, a relatively long storage life and is in a ready-to-use, pre-poured form. 30

35 The term "T.T. - Nutrient" as used herein denotes a nutrient medium comprising a reducing agent, preferably a water soluble thiosulfate in a concentration effective to inactivate growth inhibiting hydrogen peroxide, α -lipoic acid in a concentration effective to promote growth of *Streptococcus faecium*, pimaricine in a concentration effective to inhibit the growth of *Candida*, a 2,3,5-triphenyl-tetrazolium salt in a concentration effective to be reduced by *Streptococcus faecalis* to red or reddish brown formazan, and a water soluble e.g. alkali metal azide in a concentration effective to inhibit growth of Gram-negative flora but to allow uninhibited growth of *Enterococci*. The composition of the medium may be such that *Streptococcus faecalis* of serologic group D incubated on the said medium produces red to reddish brown, completely flat and small colonies with a metallic gloss on the said medium. 40

The water soluble thiosulfate is conveniently sodium thiosulfate, the 2,3,5-triphenyltetrazolium salt is conveniently 2,3,5-triphenyltetrazolium chloride and the alkali metal azide is conveniently sodium azide.

45 In a preferred embodiment "T.T - nutrient" comprises tryptose, yeast extract, glucose, disodium hydrogen phosphate, sodium thiosulfate, α -lipoic acid, agar-agar, pimaricine, 2,3,5-triphenyltetrazolium chloride, sodium azide and deionised or distilled water and in an especially preferred embodiment has the composition: 45

50	Tryptose	about 20.0 g	50
	yeast extract	about 5.0 g	
	glucose	about 2.0 g	
	disodium hydrogen phosphate	about 4.0 g	
	sodium thiosulfate	about 0.3g	
55	α -lipoic acid	about 0.001 g	55
	Agar-agar	about 15.0 g	
	pimaricine	about 0.1 g	
	2,3,5-triphenyltetrazolium chloride (T.T.C)	about 0.1 g	
60	sodium azide	about 0.8 g	60

per litre of deionized or distilled water. The pH of the nutrient medium is about 7.2.

65 The water soluble thiosulfate e.g. sodium thiosulfate inactivates the growth-inhibiting hydrogen peroxide, since the *Enterococci* do not possess any catalase with which to break H₂O₂ down. 65

α -lipoic acid in the above-mentioned concentration serves as a growth promotor for *Streptococcus faecium*.

Pimaricine in the above-indicated concentration inhibits the growth of *Candida* and other yeasts and mould fungi.

5 T.T. e.g. T.T.C. is reduced by *Streptococcus faecalis* to the red to reddish-brown colour of formazan. 5

The alkali metal e.g. sodium azide in the above-mentioned concentration inhibits only the growth of the Gram-negative accompanying flora and allows uninhibited growth of the *Enterococci*.

10 The term "Peptone-thiocyanate nutrient" as used herein denotes a nutrient medium comprising a vegetable protein hydrolysate (peptone) in a concentration effective to enhance the growth of *Candida*, a surfactant in a concentration effective to suppress the growth of Gram-positive microorganisms and a water soluble thiocyanate in a concentration effective to suppress the growth of Gram-negative accompanying microorganisms; the composition of the medium being such that pathogens of the genera *Candida* incubated in the said medium grow in colonies of a dirty white colour. 10 15

The vegetable protein hydrolysate (peptone) for use in the "Peptone-thiocyanate nutrient" medium of the present invention is preferably that available under the trade name Mycopeptone B.

20 The water soluble thiocyanate may, for example, be sodium thiocyanate and the surfactant is generally an anionic surfactant, especially a long chain alkyl sulphate such as heptadecyl sulfate preferably used as its salt e.g. sodium heptadecyl sulfate. 20

The pH of the nutrient medium is preferably 5.5 to 6.0 especially about 5.8.

25 In a preferred embodiment "Peptone-thiocyanate-nutrient" comprises a vegetable protein hydrolysate, liver hydrolysate, glucose, yeast extract, sodium chloride, magnesium sulfate, manganese (II) chloride, sodium heptadecyl sulfate, potassium thiocyanate, agar-agar and deionised or distilled water and in an especially preferred embodiment has the following composition: 25

30	Vegetable protein hydrolysate	about 15.0 g	30
	liver hydrolysate	about 1.0 g	
	glucose	about 5.0 g	
	yeast extract	about 3.0 g	
	sodium chloride	about 3.0 g	
35	magnesium sulfate heptahydrate	about 0.25 g	35
	manganese (II) chloride tetrahydrate	about 0.001 g	
	sodium heptadecyl sulfate (Tergitol 7)	about 0.05 g	
	potassium thiocyanate	about 20.0 g	
	agar-agar	about 22.0 g	

40 per litre of deionised or distilled water. The pH of the nutrient medium is about 5.8. 40

Characteristics

45 Vegetable protein hydrolysates, especially Mycopeptone B mainly enhances the growth of *Candida* and other yeast strains. 45

A surfactant e.g. sodium heptadecyl sulfate (Tergitol 7) suppresses the growth of all Gram-positive microorganisms in this concentration and at this pH-value.

50 A water soluble thiocyanate e.g. potassium thiocyanate suppresses the growth of all Gram-negative accompanying organisms in the above-mentioned concentration and at the above-mentioned pH-value. 50

55 As described above, the method of the present invention is particularly concerned with the determination of the types of pathogens causing infections of the urogenital tract. The method of the present invention may however be employed for the examination of other biological material, in which case it may be necessary to include the use of further nutrient media, for example, for examination of faeces in order to identify *Salmonella* or *Shigella*. The method of the present invention may also be extended to the identification of the particular species concerned by including further nutrient media in the investigation process. 55

60 The method of the present invention is conveniently effected by inoculating each nutrient medium with a urine sample, the pH of the urine sample having been measured prior to inoculation. Thus the method of the present invention may, for example, be effected as follows:- 60

65 the temperature of the cooled nutrient media is allowed to rise to room temperature. The pH-value of the middle portion of the morning urine is measured. An inoculation loop filled with the urine sample is applied and spread on each nutrient medium except that the sample 65

is only spotted on DNase nutrient medium in lines in the middle of the plate. The nutrient media are then incubated for 24 hours at about 37°C a lid being placed over each nutrient medium.

Evaluation is effected as follows:—

- 5 For identification of the pathogen genera the following criteria are decisive for each individual type; the colour changes described below relate in general to the preferred pH indicators. Other indicator compounds will of course produce different hues. 5
- Citrobacter*
- 10 a) In Lactose-P-nutrient (as hereinbefore defined), lactose is in general, fermented slowly; the nutrient medium turns blue to blue-green within 24 hours. 10
b) in citrate-G-nutrient (as hereinbefore defined) the citrate is attacked; the nutrient becomes raspberry red.
- Enterobacter*
- 15 a) In Lactose-P-nutrient (as hereinbefore defined), lactose is fermented rapidly; the nutrient turns yellow-greenish and the colonies are big and slimy. 15
b) In citrate-G-nutrient (as hereinbefore defined) the citrate is attacked, the nutrient becomes raspberry red.
- Escherichia*
- 20 a) In Lactose-P-nutrient (as hereinbefore defined) the lactose is fermented rapidly, the nutrient medium becomes mandarine yellow and the colonies are not slimy. 20
b) In citrate-G-nutrient (as hereinbefore defined) the citrate is attacked. In the presence of yeast extract + glucose (0.25 + 0.05 g/ltr) there may be only a very weak growth. The nutrient turns to a light pink to orange. 25
- Klebsiella*
- 30 a) In Lactose-P-nutrient (as hereinbefore defined) the lactose is quickly fermented, in a similar manner to *Enterobacter*; the nutrient medium becomes yellow-greenish as with *Enterobacter*, or sometimes mandarine yellow as with *Escherichia*; the colonies are big and slimy as with *Enterobacter*. As opposed to *Enterobacter* and *Escherichia*, *Klebsiella* is not mobile. Mobility must therefore be checked, for example in hanging drops. 30
b) In citrate-G-nutrient (as hereinbefore defined) the citrate is attacked; the nutrient medium turns raspberry red. 35
- Pseudomonas*
- Cadmium nutrient (as hereinbefore defined) turns greenish to yellow; fluorescent pigments (pyoverdin) are formed.
- Proteus*
- 40 On obtaining a good growth on phenylalanine-G-nutrient (as hereinbefore defined) a solution of a ferric salt e.g. ferric chloride for example approximately 0.3 ml of an iron (III) - chloride solution is added to the nutrient medium. After no more than 3 minutes *Proteus* will show a typical green colouring of the nutrient medium. 45
- All species of the genus *Proteus* produce the enzyme urease, which decomposes urea into ammonia. The production of ammonia shifts the pH-value to the alkaline end of the pH range (8.2 to 9.0) and in order to detect the pH change the pH of the urine sample should be measured.
- Providencia*
- 50 On obtaining a good growth on phenylalanine-G-nutrient (as hereinbefore defined) a solution of a ferric salt e.g. ferric chloride for example approximately 0.3 ml of an iron (III) - chloride solution is added to the nutrient medium. After no more than 3 minutes, *Providencia* shows the same typical colour reaction as *Proteus*. As opposed to *Proteus*, however, the pH-value of the urine is shifted only slightly. 55
- Serratia*
- 60 *Serratia marcescens* often forms on DNase-nutrient (as hereinbefore defined) pink to cherry red pigments. On visible growth upon DNase-nutrient (as hereinbefore defined) a dilute hydrochloric acid e.g. approximately 0.3 ml of 1 N hydrochloric acid is added to the medium and after several minutes a distinctly clear zone is formed around the growth, surrounded by a turbid precipitation. *Serratia marcescens* is 96.7% DNase-positive. *Serratia liquefaciens*, playing only a subordinate role with respect to urinary tract infections, is 69.4% DNase-positive. Rods occurring with infections of the urinary tract are able to grow 65

on this nutrient medium, however, they are DNase negative.

Staphylococcus

In Mannitol-thiocyanate-nutrient the mannitol is degraded by *Staphylococcus aureus*, *Staphylococcus epidermidis* biotype 4 and several *Staphylococci saprophyticus* strains. Acid is produced as a result of this process and may be detected by a colour change if a pH-indicator is used. The nutrient medium becomes reddish-yellow if phenol red is used as indicator. When prolonging incubation to 36 hours the colonies become bright yellow.

In suspicious cases it is recommended to carry out the quick coagulase test. *Staphylococcus aureus* as opposed to other *Staphylococci* - is coagulase-positive.

Streptococcus (Enterococcus)

Streptococcus faecalis of the serologic group D reduces T.T. to formazan and grows in red to reddish-brown, completely flat and small colonies with a metallic gloss on the T.T. nutrient (as hereinbefore defined).

Streptococcus faecium of the serologic group D - apart from the sub-genus *casseliflavus* - is not capable of reducing T.T. to formazan. The colonies are colourless, completely flat and small.

There are types of the genera *Proteus* and *Serratia*, which occasionally grow on T.T.-nutrient in single colonies, which are larger, elevated and red without metallic gloss. These are mobile, as opposed to the above-mentioned *Enterococci*. It is therefore recommended that mobility is examined in suspicious cases, for example in hanging drops.

Candida

On peptone-thiocyanate-nutrient *Candida* grows in colonies of a dirty white colour.

According to a further feature of the present invention there is provided a kit for determining the genera of pathogens capable of causing urogenital tract infections which comprises in combination each of the following nutrient media:-

- Citrate-G-nutrient (as herein defined)
- Phenylalanine-lithium-G-nutrient (as herein defined)
- Mannitol-thiocyanate nutrient (as herein defined)
- Peptone-thiocyanate nutrient (as herein defined)

each of said nutrient media being retained in a sterile container.

The kit preferably also contains Lactose-P-nutrient (as herein defined), Cadmium nutrient (as herein defined), DNase nutrient (as herein defined) and/or T.T.-nutrient (as herein defined).

In one embodiment of the present invention the kit comprises a plurality of sterile containers e.g. of plastics material preferably formed by deep drawing, each container being provided with a closure member for sealing the container and each container having therein one of the above-mentioned nutrient media.

In a further embodiment of the present invention the kit comprises a holder divided into a plurality of compartments adapted to receive a plurality of different nutrient media, the nutrient media being retained in sterile condition in the holder. In this connection the present invention also relates to a kit in which the nutrient media are contained in individual sterile sealed packages in which case each package may, for example, be retained in the abovementioned holder.

It is convenient to fill the sterilized nutrient media into the lower part of sterile deep-drawn containers equipped with a tightly sealing lid or other closure means. The lower part of these containers is divided into eight square cavities e.g. arranged in two columns of four, the base of each cavity being, for example, 8 to 10cm² and the depth of each cavity being 1 to 1.5 cms. Several mls, preferably about 5 or 6 mls of the above-mentioned nutrient media, is conveniently placed in each cavity.

The kit of the present invention may, of course, be employed in the method of the present invention providing all eight of the above-mentioned nutrient media are present. The preparation of the various nutrient media referred to in the specification is illustrated in the following Examples:-

Example 1

Lactose-P-Nutrient

Composition

	Lactose	10.0 g	
	polypeptone	5.0 g	
5	yeast extract	3.0 g	5
	sodium heptadecyl sulfate (Tergitol 7)	0.15 g	
	pimaricine	0.1 g	
	bromthymol blue	0.025 g	
	agar-agar	18.0 g	
10	distilled water	1 litre	10
	final pH-value: 6.9 ± 0.1		

Preparation

15	36.18 g of the above-mentioned mixture, except for pimaricine, are suspended in 900 ml of distilled water, steeped, thoroughly mixed with the aid of a magnetic stirrer and subsequently heated on a boiling water-bath for approximately 10 to 15 minutes. The suspension is cooled to 50°C and the pH-value is measured (6.8 ± 0.1). The nutrient medium is then sterilized in an autoclave at 121°C for 15 minutes, cooled to 55°C and mixed with 100 ml of a 0.1% sterile-filtered pimaricine solution, thoroughly mixed with the aid of a magnetic stirrer. The nutrient medium may then be dispensed into sterile deep-drawn containers, 5.5 to 6.0 mls of nutrient medium being dispensed into each container. The container may now be sealed with a tightly fitting lid.	15
20		20

Example 2

25		25
	<i>Citrate-G-Nutrient</i>	

Composition

30	Trisodium citrate dihydrate	5.0 g	30
	mixture of salts of bile acids	1.0 g	
	pimaricine	0.1 g	
	glucose	0.05 g	
	yeast extract	0.25 g	
35	sodium chloride	5.0 g	35
	magnesium sulfate heptahydrate	0.2 g	
	ammonium dihydrogen phosphate	0.2 g	
	sodium ammonium hydrogen phosphate tetrahydrate	0.8 g	
	phenol red	0.02 g	
40	agar-agar	15.0 g	40
	distilled water	1 litre	
	final pH-value 7.1 ± 0.1		

Preparation:

45	27.52g of the above-mentioned mixture, except for pimaricine, are suspended in 900 ml of distilled water, steeped and thoroughly mixed with the aid of a magnetic stirrer and, subsequently, heated for approximately 10 to 15 minutes on a boiling water-bath. The suspension is cooled to 50°C and the pH-value is measured (7.3 ± 0.1). The nutrient medium is then sterilized at 121°C in an autoclave for 15 minutes, cooled to 55°C and thoroughly mixed with 100 ml of a 0.1% pimaricine solution, with the aid of a magnetic stirrer. The nutrient medium may then be dispensed into sterile deep-drawn containers, 5.5 to 6.0 mls of nutrient medium being dispensed into each container. The container may now be sealed with a tightly fitting lid.	45
50		50
55		55

*Example 3**Cadmium Nutrient*

60	<i>Composition</i>	60
	Proteose peptone	20.0 g
	sodium chloride	1.2 g
	dipotassium hydrogen phosphate	0.8 g
65	potassium nitrate	0.6 g
		65

	calcium nitrate tetrahydrate	0.4 g	
	magnesium sulfate heptahydrate	0.6 g	
	cadmium sulfate octahydrate	0.005 g	
	glycerin	8.0 ml	
5	N-alkyl-aminocrotonic acid Ampholyte KKPK 55	0.5 g	5
	agar-agar	15.0 g	
	distilled water	1 litre	
	final pH-value: 7.2 ± 0.1	1 litre	
10	<i>Preparation:</i>		10
	0.50 g of Ampholyte KKPK 55 and 8 ml of glycerin are well suspended in 1000 ml of distilled water with the aid of a magnetic stirrer. The suspension is mixed with 48.6g of the remaining components of the nutrient medium and then heated for 10 to 15 minutes on a boiling water-bath. The mixture is cooled to 50°C and the pH-value is measured (6.9 ± 0.1). The nutrient medium is then sterilized for 15 minutes at 121°C in an autoclave and cooled to 50°C.		
15	The nutrient medium may then be dispensed into sterile deep-drawn containers, 5.5 to 6.0 mls of nutrient medium being dispensed into each container. The container may now be sealed with a tightly fitting lid.		
20			
	<i>Example 4</i>		25
25	<i>Phenylalanine-lithium-G-Nutrient</i>		
	<i>Composition</i>		
30	Proteose peptone	10.0 g	30
	yeast extract	3.0 g	
	sodium chloride	5.0 g	
	disodium hydrogen phosphate	5.6 g	
	potassium hydrogen phosphate	0.2 g	
35	L-phenylalanine	2.0 g	35
	mixture of salts of bile acids	1.0 g	
	lithium chloride	2.0 g	
	pimaricine	0.2 g	
	agar-agar	16.0 g	
40	distilled water	1 litre	40
	final pH-value: 7.2 ± 0.1		
	<i>Preparation</i>		
45	44.8 g of the above-mentioned mixture, except for pimaricine, are thoroughly suspended in 900 ml of distilled water with the aid of a magnetic stirrer and heated for 10 to 15 minutes on a boiling water-bath. The suspension is cooled to 50°C and the pH-value is measured (7.3 ± 0.1). The nutrient medium is then sterilized at 121°C in an autoclave for 15 minutes, cooled to 55°C and thoroughly mixed with 100 ml of a 0.2% sterile filtered pimaricine solution, with the aid of a magnetic stirrer.		
50	The nutrient medium may then be dispensed into sterile deep-drawn containers, 5.5 to 6.0 mls of nutrient medium being dispensed into each container. The container may now be sealed with a tightly fitting lid.		
55	<i>Example 5</i>		55
	<i>DNase Nutrient</i>		
	<i>Compositon</i>		
60	Tryptose	16.0 g	60
	desoxyribonucleinic acid	1.6 g	
	sodium chloride	4.0 g	
	N-alkyl-aminocrotonic acid		
65	Ampholyte KKPK 55	0.15g	65

	methyl green	0.04 g	
	agar-agar	12.0 g	
	distilled water	1 litre	
5	final pH-value 7.3 ± 0.1		5
	<i>Preparation</i>		
10	0.15 g of Ampholyte KKPK 55 are thoroughly suspended in 1000 ml of distilled water with the aid of a magnetic stirrer, mixed with 33.6 g of the remaining substances, and then heated for 10 to 15 minutes on a boiling water-bath. The suspension is cooled to 50°C and the pH-value measured (7.0 ± 0.1). The nutrient medium is then sterilized for 15 minutes in an autoclave and cooled to 50°C.		
15	The nutrient medium may then be dispensed into sterile deep-drawn containers, 5.5 to 6.0 mls of nutrient medium being dispensed into each container. The container may now be sealed with a tightly fitting lid.		
	<i>Example 6</i>		
	<i>Mannitol-Thiocyanate Nutrient</i>		
20	<i>Composition</i>		
	Casein peptone	10.0 g	
	meat extract	5.0 g	
25	gelatin	5.0 g	25
	D-mannitol	10.0 g	
	yeast extract	5.0 g	
	sodium pyruvate	10.0 g	
	disodium hydrogen phosphate	6.0 g	
30	lithium chloride	5.0 g	30
	sodium chloride	5.0 g	
	potassium thiocyanate	30.0 g	
	phenol red	0.02 g	
	pimaricine	0.2 g	
35	agar-agar	16.0 g	35
	distilled water	1 litre	
	final pH-value 7.2 ± 0.1		
	<i>Preparation</i>		
40	107.02 g of the dehydrated nutrient medium are thoroughly suspended in 900 ml of distilled water with the aid of a magnetic stirrer and heated for approximately 10 to 15 minutes on a boiling water-bath. The suspension is cooled to 50°C and the pH-value is adjusted by means of a 10% sodium hydroxide solution to 7.4. The nutrient medium is then sterilized in an autoclave at 121°C for 15 minutes, cooled to 50°C and then mixed with 100 mls of a 0.2% solution of pimaricine in water.		
45	The nutrient medium may then be dispensed into sterile deep-drawn containers, 5.5 to 6.0 mls of nutrient medium being dispensed into each container. The container may now be sealed with a tightly fitting lid.		
50	<i>Example 7</i>		
	<i>T.T.C. - Nutrient</i>		
55	<i>Composition</i>		
	Tryptose	20.0 g	
	yeast extract	5.0 g	
	glucose	2.0 g	
60	disodium hydrogen phosphate	4.0 g	60
	sodium thiosulfate	0.3 g	
	α -lipoic acid	0.001 g	
	Agar-Agar	15.0 g	
	pimaricine	0.1 g	
65	2,3,5-triphenyltetrazolium chloride (T.T.C.)	0.1 g	65
	sodium azide	0.8 g	

distilled water
final pH-value: 7.2 \pm 0.1

1 litre

Preparation

- 5 46.3g of the above-mentioned mixture, except for pimaricine, 2,3,5-triphenyltetrazolium chloride and sodium azide, are suspended in 890 ml of distilled water, steeped and thoroughly mixed with the aid of a magnetic stirrer. The mixture is then heated for 10 to 15 minutes on a boiling water-bath. The suspension is cooled to 50°C and the pH-value adjusted to 7.2. Subsequently, the medium is sterilized for 15 minutes at 121°C in an autoclave and cooled to 50°C. The following solutions, in sterile filtered condition, are then added: 100 ml of a 0.1% pimaricine solution, 2.5 ml of a 4% T.T.C.-solution and 10 ml of a 4% sodium azide solution. All solutions should be prepared 30 minutes before addition to the remaining nutrient medium constituents at the outside, and should be shelved protected from the light. The mixture is stirred thoroughly with a magnetic stirrer.

15 The nutrient medium may then be dispensed into sterile deep-drawn containers, 5.5 to 6.0 mls of nutrient medium being dispensed into each container. The container may now be sealed with a tightly fitting lid.

Example 8

Peptone-Thiocyanate Nutrient

Composition

- 25 Vegetable protein hydrolysate (Mycopeptone B) 15.0 g
liver hydrolysate 1.0 g
glucose 5.0 g
30 yeast extract 3.0 g
sodium chloride 3.0 g
magnesium sulfate heptahydrate 0.25 g
manganese (II) chloride tetrahydrate 0.001 g
sodium heptadecyl sulfate (Tergitol 7) 0.05 g
35 potassium thiocyanate 20.0 g
agar-agar 22.0 g
distilled water 1 litre
final pH-value: 5.8 \pm 0.1.

Preparation

- 40 69.3 g of the above-mentioned mixture are suspended in 1 litre of distilled water, steeped, mixed together with the aid of a magnetic stirrer and subsequently heated on a boiling water-bath for 10 to 15 minutes. The solution is cooled to 50°C and the pH-value adjusted to 5.8 \pm 0.1. The medium is then sterilized at 121°C in an autoclave for 15 minutes, and cooled to 50°C.

45 The nutrient medium may then be dispensed into sterile deep-drawn containers, 5.5 to 6.0 mls of nutrient medium being dispensed into each container. The container may now be sealed with a tightly fitting lid.

- 50 The pimaricine contained in some of the media described herein may, of course, be replaced wholly or in part by another material with similar characteristics of inhibiting *Candida* and other yeasts and mould fungi while permitting the growth of the microorganisms to be tested for.

WHAT WE CLAIM IS:

- 55 1. A method for determining the genera of pathogens capable of causing urogenital tract infections which comprises inoculating each of the following nutrient media:
Lactose-P-Nutrient (as herein defined),
Citrate-G-Nutrient (as herein defined),
60 Cadmium Nutrient (as herein defined),
Phenylalanine-lithium-G-Nutrient (as herein defined),
DNase Nutrient (as herein defined),
Mannitol-Thiocyanate Nutrient (as herein defined),
T.T. - Nutrient (as herein defined)
65 Peptone-Thiocyanate Nutrient (as herein defined),

- with a sample of the said pathogen, incubating each of the nutrient media, and determining the genera of the said pathogen by evaluation of the growth if any on each of the nutrient media.
2. A method as claimed in claim 1 wherein Lactose-P-nutrient (as herein defined) contains bromothymol blue as indicator. 5
3. A method as claimed in claim 1 or claim 2 wherein Lactose-P-nutrient (as herein defined) contains an anionic long chain alkyl sulfate as the anionic surfactant.
4. A method as claimed in claim 3 wherein the Lactose-P-nutrient comprises a heptadecyl sulfate as anionic surfactant.
5. A method as claimed in claim 4 wherein the heptadecyl sulfate is sodium heptadecyl sulfate. 10
6. A method as claimed in any one of the preceding claims wherein Lactose-P-nutrient comprises lactose, polypeptone, yeast extract, sodium heptadecyl sulfate, pimaricine, bromothymol blue, agar-agar and deionized or distilled water.
7. A method as claimed in claim 6 wherein Lactose-P-nutrient has the composition:- 15
- | | | |
|--|---------------|----|
| Lactose | about 10.0 g | 15 |
| polypeptone | about 5.0 g | |
| yeast extract | about 3.0 g | |
| sodium heptadecyl sulfate | about 0.15 g | |
| pimaricine | about 0.1 g | 20 |
| bromothymol blue | about 0.025 g | |
| agar-agar | about 18.0 g | |
| per litre of deionised or distilled water. | | |
8. A method as claimed in any one of the preceding claims wherein Citrate-G-nutrient (as herein defined) additionally comprises yeast extract and glucose. 25
9. A method as claimed in claim 8 wherein the water-soluble citrate is sodium or potassium citrate.
10. A method as claimed in claim 9 wherein the water-soluble citrate is trisodium citrate dihydrate.
11. A method as claimed in any one of the preceding claims wherein Citrate-G-nutrient (as herein defined) contains phenol red as indicator. 30
12. A method as claimed in any one of the preceding claims wherein Citrate-G-nutrient (as herein defined) comprises trisodium citrate dihydrate, a mixture of salts of bile acids, pimaricine, glucose, yeast extract, sodium chloride, hydrated magnesium sulfate, ammonium dihydrogen phosphate, hydrated sodium ammonium hydrogen phosphate, phenol red, agar-agar and deionised or distilled water. 35
13. A method as claimed in claim 12 wherein Citrate-G-nutrient (as herein defined) has the composition:-
- | | | |
|---|--------------|----|
| Trisodium citrate dihydrate | about 5.0 g | 40 |
| mixture of salts of bile acids | about 1.0 g | |
| pimaricine | about 0.1 g | |
| glucose | about 0.05 g | |
| yeast extract | about 0.25 g | |
| sodium chloride | about 5.0 g | 45 |
| magnesium sulfate heptahydrate | about 0.2 g | |
| ammonium dihydrogen phosphate | about 0.2 g | |
| sodium ammonium hydrogen phosphate tetrahydrate | about 0.8 g | |
| phenol red | about 0.02 g | 50 |
| agar-agar | about 15.0 g | |
| per litre of deionized or distilled water | | |
14. A method as claimed in any one of the preceding claims wherein the Cadmium nutrient (as herein defined) comprises cadmium sulfate.
15. A method as claimed in claim 14 wherein the Cadmium nutrient (as herein defined) comprises proteose peptone, sodium chloride, dipotassium hydrogen phosphate, potassium nitrate, hydrated calcium nitrate, hydrated magnesium sulfate, hydrated cadmium sulfate, glycerin, an N-alkyl-aminocrotonic acid, agar-agar and deionised or distilled water. 55
16. A method as claimed in claim 15 wherein Cadmium nutrient (as herein defined) has the composition:- 60
- | | | |
|--------------------------------|--------------|----|
| Proteose peptone | about 20.0 g | |
| sodium chloride | about 1.2 g | |
| dipotassium hydrogen phosphate | about 0.8 g | |
| potassium nitrate | about 0.6 g | 65 |

- | | | | |
|----|---|--------------|----|
| | calcium nitrate tetrahydrate | about 0.4 g | |
| | magnesium sulfate heptahydrate | about 0.6 g | |
| | cadmium sulfate octahydrate | about 0.005g | |
| | glycerin | about 8.0 ml | |
| 5 | N-alkyl-aminocrotonic acid | about 0.5 g | 5 |
| | agar-agar | about 15.0 g | |
| | per litre of deionised or distilled water. | | |
| | 17. A method as claimed in any one of the preceding claims wherein Phenylalanine-lithium-G-nutrient (as herein defined) comprises L-phenylalanine. | | |
| 10 | 18. A method as claimed in claim 17 wherein the water-soluble lithium salt is lithium chloride. | | 10 |
| | 19. A method as claimed in claim 18 wherein Phenylalanine-lithium-G-nutrient (as herein defined), comprises proteose peptone, yeast extract, sodium chloride, disodium hydrogen phosphate, potassium hydrogen phosphate, L-phenylalanine, a mixture of salts of bile acids, lithium chloride, pimaricine, agar-agar and deionised or distilled water. | | |
| 15 | 20. A method as claimed in claim 19 wherein Phenylalanine-lithium-G-nutrient (as herein defined) has the composition:- | | 15 |
| | Proteose peptone | about 10.0 g | |
| 20 | yeast extract | about 3.0 g | 20 |
| | sodium chloride | about 5.0 g | |
| | disodium hydrogen phosphate | about 5.6 g | |
| | potassium hydrogen phosphate | about 0.2 g | |
| | L-phenylalanine | about 2.0 g | |
| 25 | mixture of salts of bile acids | about 1.0 g | 25 |
| | lithium chloride | about 2.0 g | |
| | pimaricine | about 0.2 g | |
| | agar-agar | about 16.0 g | |
| | per litre of deionised or distilled water. | | |
| 30 | 21. A method as claimed in any one of the preceding claims wherein the DNase nutrient (as herein defined) comprises an indicator. | | 30 |
| | 22. A method as claimed in claim 21 wherein the indicator is methyl green. | | |
| | 23. A method as claimed in any one of the preceding claims wherein DNase-nutrient (as herein defined) comprises tryptose, desoxyribonucleic acid, sodium chloride, an N-alkyl-amino-crotonic acid, agar-agar and deionised or distilled water. | | |
| 35 | 24. A method as claimed in claim 23 wherein DNase nutrient (as herein defined) has the composition:- | | 35 |
| | Tryptose | about 16.0 g | |
| 40 | desoxyribonucleic acid | about 1.6 g | 40 |
| | sodium chloride | about 4.0 g | |
| | N-alkyl-aminocrotonic acid | about 0.15 g | |
| | agar-agar | about 12.0 g | |
| | per litre of deionised or distilled water. | | |
| 45 | 25. A method as claimed in claim 24 wherein the DNase nutrient (as herein defined) contains about 0.04 g of methyl green per litre of deionised or distilled water. | | 45 |
| | 26. A method as claimed in any of the preceding claims wherein Mannitol-thiocyanate nutrient (as herein defined) contains a water-soluble lithium salt. | | |
| 50 | 27. A method as claimed in any one of the preceding claims wherein Mannitol thiocyanate nutrient (as herein defined) contains a water-soluble potassium salt. | | 50 |
| | 28. A method as claimed in any one of the preceding claims wherein Mannitol-thiocyanate nutrient (as herein defined) contains a water-soluble chloride. | | |
| | 29. A method as claimed in any one of the preceding claims wherein Mannitol-thiocyanate nutrient (as herein defined) contains potassium thiocyanate. | | |
| 55 | 30. A method as claimed in any one of the preceding claims wherein Mannitol-thiocyanate nutrient (as herein defined) contains a water-soluble sodium salt. | | 55 |
| | 31. A method as claimed in any one of the preceding claims wherein Mannitol-thiocyanate nutrient (as herein defined) contains casein peptone, meat extract, gelatin, D-mannitol, yeast extract, sodium pyruvate, disodium hydrogen phosphate, lithium chloride, sodium chloride, potassium thiocyanate, a pH indicator, pimaricine, agar-agar and deionised or distilled water. | | |
| 60 | 32. A method as claimed in any one of the preceding claims wherein the pH indicator in Mannitol-thiocyanate nutrient (as herein defined) is phenol red. | | 60 |
| | 33. A method as claimed in claims 31 or 32 wherein Mannitol-thiocyanate nutrient (as herein defined) has the composition:- | | |
| 65 | | | 65 |

	Casein peptone	about 10.0 g	
	meat extract	about 5.0 g	
	gelatin	about 5.0 g	
	D-mannitol	about 10.0 g	
5	yeast extract	about 5.0 g	5
	sodium pyruvate	about 10.0 g	
	disodium hydrogen phosphate	about 6.0 g	
	lithium chloride	about 5.0 g	
	sodium chloride	about 5.0 g	
10	potassium thiocyanate	about 30.0 g	10
	phenol red	about 0.02 g	
	pimaricine	about 0.2 g	
	agar-agar	about 16.0 g	
	per litre of deionised or distilled water.		
15	34. A method as claimed in any one of the preceding claims wherein T.T.-nutrient contains a reducing agent.		15
	35. A method as claimed in claim 34 wherein T.T.-nutrient (as herein defined) contains sodium thiosulfate.		
20	36. A method as claimed in any one of the preceding claims wherein T.T.-nutrient (as herein defined) contains 2,3,5-triphenyltetrazolium chloride.		20
	37. A method as claimed in any one of the preceding claims wherein T.T.-nutrient (as herein defined) contains an alkali metal azide.		
	38. A method as claimed in claim 37 wherein the alkali metal azide is sodium azide.		
25	39. A method as claimed in any one of the preceding claims wherein T.T.-nutrient contains tryptose, yeast extract, glucose, disodium hydrogen phosphate, sodium thiosulfate, α -lipoic acid, agar-agar, pimaricine, 2,3,5-triphenyltetrazolium chloride, sodium azide and deionised or distilled water.		25
	40. A method as claimed in claim 39 wherein T.T.-nutrient has the composition:-		
30	Tryptose	about 20.0 g	30
	yeast extract	about 5.0 g	
	glucose	about 2.0 g	
	disodium hydrogen phosphate	about 4.0 g	
	sodium thiosulfate	about 0.3 g	
35	α -lipoic acid	about 0.001 g	35
	agar-agar	about 15.0 g	
	pimaricine	about 0.1 g	
	2,3,5-triphenyltetrazolium chloride (T.T.C)	about 0.1 g	
40	sodium azide	about 0.8 g	40
	per litre of deionised or distilled water.		
	41. A method as claimed in any one of the preceding claims wherein Peptone-thiocyanate nutrient (as herein defined) contains sodium thiocyanate.		
45	42. A method as claimed in any one of the preceding claims wherein Peptone-thiocyanate nutrient (as herein defined) contains an anionic long chain alkyl sulfate as surfactant.		45
	43. A method as claimed in claim 42 wherein Peptone-thiocyanate nutrient (as herein defined) contains a heptadecyl sulfate as an anionic surfactant.		
50	44. A method as claimed in claim 43 wherein the heptadecyl sulfate is sodium heptadecyl sulfate.		50
	45. A method as claimed in any one of the preceding claims wherein the pH of Peptone-thiocyanate nutrient (as herein defined) is about 5.8.		
55	46. A method as claimed in any one of the preceding claims wherein Peptone-thiocyanate nutrient (as herein defined) contains vegetable protein hydrolysate, liver hydrolysate, glucose, yeast extract, sodium chloride, magnesium sulfate, manganese (II) chloride, sodium heptadecyl sulfate, potassium thiocyanate, agar-agar and deionised or distilled water.		55
	47. A method as claimed in claim 46 wherein Peptone-thiocyanate nutrient (as herein defined) has the composition:-		
60	Vegetable protein hydrolysate	about 15.0 g	60
	liver hydrolysate	about 1.0 g	
	glucose	about 5.0 g	
	yeast extract	about 3.0 g	
65	sodium chloride	about 3.0 g	65

	magnesium sulfate heptahydrate	about 0.25 g	
	manganese (II) chloride tetrahydrate	about 0.001 g	
	sodium heptadecyl sulfate	about 0.05 g	
	potassium thiocyanate	about 20.0 g	
5	agar-agar	about 22.0 g	5
	per litre of deionised or distilled water.		
	48. A method as claimed in any one of claims 17-20 wherein the pathogens <i>Proteus</i> and <i>Providencia</i> are identified by the phenylalanine desaminase test on the incubated sample of pathogen.		
10	49. A method as claimed in claim 48 wherein the pathogens <i>Proteus</i> and <i>Providencia</i> are distinguished by pH measurement in order to detect presence of ammonia.		10
	50. A method as claimed in any one of claims 21-25 wherein the pathogen <i>Serratia</i> is identified by adding dilute acid to the incubated nutrient medium.		
15	51. A method as claimed in any one of the preceding claims substantially as herein described.		15
	52. A kit for determining the genera of pathogens capable of causing urogenital tract infections which comprises in combination each of the following nutrient media:-		
	Citrate-G-nutrient (as herein defined)		
20	Phenylalanine-lithium-G-nutrient (as herein defined)		20
	Mannitol-thiocyanate nutrient (as herein defined), and		
	Peptone-thiocyanate nutrient (as herein defined)		
	each of said nutrient media being retained in a sterile container.		
25	53. A kit as claimed in claim 52 which also contains Lactose-P-nutrient (as herein defined), Cadmium-nutrient (as herein defined), DNase nutrient (as herein defined) and/or T.T.-nutrient (as herein defined).		25
	54. A kit as claimed in claim 52 or claim 53 which comprises a plurality of sterile containers, each container being provided with a closure member for sealing the container and each container having therein one of the said nutrient media.		30
30	55. A kit as claimed in claim 54 wherein the container is of a plastics material.		
	56. A kit as claimed in claim 55 wherein the containers are formed by deep drawing.		
	57. A kit as claimed in claim 52 or claim 53 which comprises a holder divided into a plurality of compartments adapted to receive a plurality of different nutrient media, each compartment retaining a different nutrient medium in sterile condition.		35
35	58. A kit as claimed in claim 52 or claim 53 wherein each nutrient medium is contained in an individual sterile sealed package.		
	59. A kit as claimed in any one of claims 53 to 58 wherein the Lactose-P-nutrient is as defined in any one of claims 2-7.		
40	60. A kit as claimed in any one of claims 52 to 59 wherein the Citrate-G-nutrient is as defined in any one of claims 8-13.		40
	61. A kit as claimed in any one of claims 53 to 60 wherein the Cadmium nutrient is as defined in any one of claims 14-16.		
	62. A kit as claimed in any one of claims 52 to 61 wherein the Phenylalanine-lithium-G-nutrient is as defined in any one of claims 17-20.		45
45	63. A kit as claimed in any one of claims 53 to 62 wherein the DNase-nutrient is as defined in any one of claims 21-25.		
	64. A kit as claimed in any one of claims 52 to 63 wherein the Mannitol-thiocyanate nutrient is as defined in any one of claims 26-33.		
50	65. A kit as claimed in any one of claims 53 to 64 wherein the T.T.-nutrient is as defined in any one of claims 34-40.		50
	66. A kit as claimed in any one of claims 52 to 65 wherein the Peptone-thiocyanate nutrient is as defined in any one of claims 41-47.		
	67. A kit as claimed in claim 52 substantially as herein described.		
55	68. Citrate-G-nutrient (as herein defined).		55
	69. Phenylalanine-lithium-G-nutrient (as herein defined).		
	70. Mannitol-thiocyanate nutrient (as herein defined).		
	71. Peptone-thiocyanate nutrient (as herein defined).		
60	For the Applicants FRANK B. DEHN & CO., Chartered Patent Agents, Imperial House, 15 - 19 Kingsway, London, W.C.2.		60
65			65